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REVIEW

The use of concanavalin A to study the immunoregulation of human T cells

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INTRODUCTION

Control mechanisms in biology are impressively precise, an essential requisite when dealing with systems such as the immune response with its capacity for producing potent inflammation. In recent years the active nature of the control mechanisms which regulate both thymus-derived 'T' cell and bone marrow-derived 'B' cell effector function has been established clearly. Nature's control mechanisms for immune responses utilize her 'tug-of-war' approach, so successfully applied to the autonomic nervous system and elsewhere. Opposing forces, often simultaneously operative, produce a net immunological effect. With the introduction of antigen to the immune system, effector and suppressor cell mechanisms are activated that together decide the nature and intensity of the response. Sir Isaac Newton would no doubt have appreciated the efficiency of such a system in which for every immunological effector action there seems to be an almost equal but opposite regulatory response. Many different and complex immunoregulatory systems have already been described for both animal and human immune responses. In fact, the reader of this review may well wonder how any successful immune response ever occurs with so many suppressor influences in existence. The study of immunoregulatory mechanisms has been profitable in terms of increased understanding of both the normal physiology of the immune response and our perception of immunopathology. The readily proposed theoretical mechanisms by which defects in immunoregulatory cells might produce disease have now been established as important in numerous clinical studies. Thus immunoregulatory action can be inappropriately excessive or restrained and effector cells may be both unusually sensitive or insensitive to control mechanisms. In this review one mechanism for assaying, *in vitro*, the integrity of one subset of immunoregulatory cells—those activated by the plant mitogen concanavalin A (Con A)—will be examined. Such cells can regulate both humoral and cell-mediated immunity but such is the complexity of the subject that the regulation of humoral immune responses (reviewed by Gershon, 1974; Waldman & Broder, 1977) will not be discussed. This review concentrates on studies of the regulation of T cells by Con A-activated T suppressor cells. Studies in the mouse are reviewed before studies in normal humans. A number of the clinical studies on the immunoregulation of T cells in different disease states, thought to have an immune pathogenesis, are tabulated.

REGULATION OF MURINE T CELL RESPONSES BY CON A-ACTIVATED T CELLS

The ability of the lectin, Con A, usually extracted from the jackbean to activate suppressor cells, was first recognized in studies with mouse lymphocytes by Dutton in 1972. He reported that in addition to the well-established induction of T cell proliferation that follows the exposure of splenic

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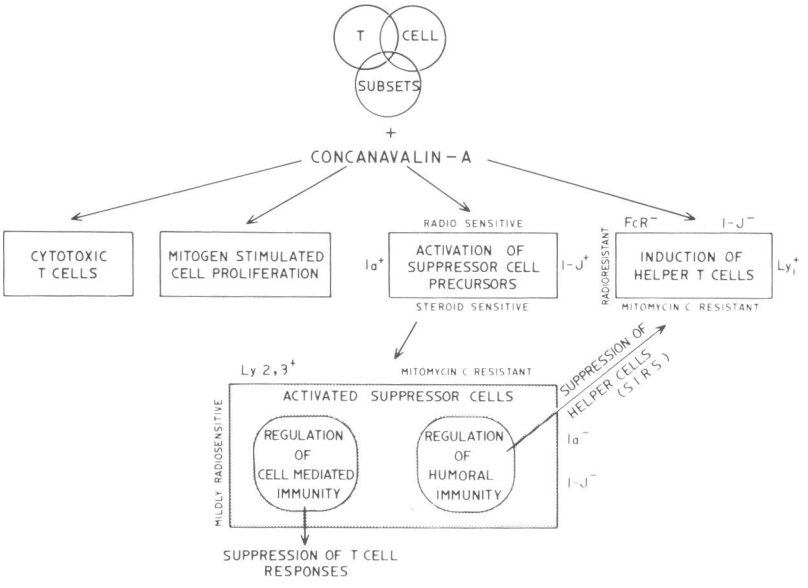

Fig. 1. T cell subsets stimulated by concanavalin A give rise to four functionally distinct T cell populations. The activation of suppressor cell precursors or promoters, leads, in turn, to active suppressor cell stimulation that results in the regulation of both B cell production of antibody (virulent effect on helper cells) and the suppression of T cell responses.

lymphocytes to Con A, both helper and suppressor cell activities were triggered. Since then, studies in the mouse have identified at least four functionally, and in some respects physically, different T cells that respond to Con A (Fig. 1).

(i) Con A activates cells capable of helping B cells respond to thymus-dependent antigens (Dutton, 1972; Tse & Dutton, 1976; Cantor & Boyse, 1975). Such cells are radiosensitive, small lymphocytes that express the Ly 1 surface antigen. They sediment slowly through a Ficoll velocity gradient and do not require the continued presence of Con A on the helper cell's membrane after initial contact (Tse & Dutton, 1977). These cells proliferate poorly after Con A activation and their helper cell function does not require DNA synthesis or cell division as the cells are resistant to mitomycin C (Tse & Dutton, 1977). Con A-susceptible helper cells do not carry receptors for the Fc portion of IgG nor do they express surface antigens coded for in the I-J subregion of the mouse's major histocompatibility complex (MHC) but may express I-A subregion antigens (Okumura *et al.*, 1976; Tada, Tanigushi & David, 1976; Tada *et al.*, 1976; Tada, 1977).

(ii) Con A activates cells that proliferate industriously as measured by ^{3}H-thymidine incorporation and cell division. These cells are denser than the helper cells and sediment deeper and more rapidly through a Ficoll velocity gradient (Tse & Dutton, 1977).

(iii) Con A can activate a subpopulation of T cells to become cytotoxic for a number of allogeneic normal and malignant cells (Bevan, 1975; MacDonald *et al.*, 1975). Helper T cells are probably necessary (Pilarski, Bretscher & Baum, 1977). Although these cells have the same density characteristics as cells that proliferate after exposure to Con A, they may be different as they become cytotoxic independent of DNA synthesis (Tse & Dutton, 1977).

(iv) Con A activates a population of suppressor-cell inducers or promoters (Niederhuber *et al.*, 1976; Frelinger, Niederhuber & Shreffler, 1976). These cells are Ly 1$^+$ and actively synthesize DNA after Con A activation. In this sense they differ from the Ly 1$^+$ helper cells. Such cells are Ia$^+$ and once activated they recruit other cells which are Ia$^-$ that subsequently proliferate. The murine suppressor cell precursors carry surface antigens coded for in the I-J region of the mouse histocompatibility complex. I-J$^+$ cells have been found to be important in immunoregulation in a number of animal systems (Tada *et al.*, 1976). Promoter cells which are quite radiosensitive (Tse & Dutton, 1977; Feldman *et al.*, 1977) appear to be resistant to cyclophosphamide but are killed by

anti-Ly 1 antisera and complement (Whisler & Stobo, 1978). After activation they in turn activate a subpopulation of suppressor T cells. For the fullest activation of these latter cells, macrophage interaction may be essential (Tadakuma & Pierce, 1976). The suppressor cells so activated are I-J, Ly $2,3^+$ well-differentiated cells which are relatively radioresistant. In such a population exist cells (perhaps distinctive) that can suppress both T and B cell responses *in vitro*. They can be recovered from the same section of a Ficoll velocity gradient occupied by proliferating cells responding to the mitogenic effect of Con A (Tse & Dutton, 1977). However, these Con A-activated suppressor cells proliferate little and at no stage of either the induction or expression of their regulatory activity is DNA synthesis or cell proliferation mandatory (Tse & Dutton, 1977). Their suppressor effects are not mediated by cytotoxicity directed towards responding cells.

Con A activates suppressor mechanisms very rapidly. Rich & Pierce (1973) showed that an inhibitory factor can be isolated by culturing spleen cells with Con A for just 12 hr. In the rabbit, Redelman *et al.* (1976) demonstrated the existence of suppressor activity in Con A-stimulated populations well before DNA synthesis commenced. Roszman (1975) observed that a 2-hr pulse of rabbit spleen cells with Con A was sufficient to activate suppressor cells without DNA synthesis.

Once in tissue culture, murine suppressor cells capable of responding to Con A may be short-lived (Dutton, 1972), although this may represent artifacts and vary with the culture conditions (Sampson, Grotelueschen & Kauffman, 1975).

Although the response to Con A is non-specific, immunoregulatory T cell mechanisms are activated by this mitogen, thus providing a phenomenon that offers an opportunity for dissection of suppressor mechanisms similar to those offered by mitogen-stimulated cell proliferation in analysing effector responses. After the initial reports of suppressor cell activation by Con A in the mouse the system was soon adapted with success to human studies. As in the mouse, Con A-activated cells were found to suppress both B and T cell responses.

REGULATION OF HUMAN T CELL RESPONSES BY CON A-ACTIVATED T CELLS

The picture of immunoregulation in humans is certainly no less complex than that of mice. Fig. 2 illustrates some of this complexity. Peripheral T lymphocyte subsets (Broder *et al.*, 1978; Stobo,

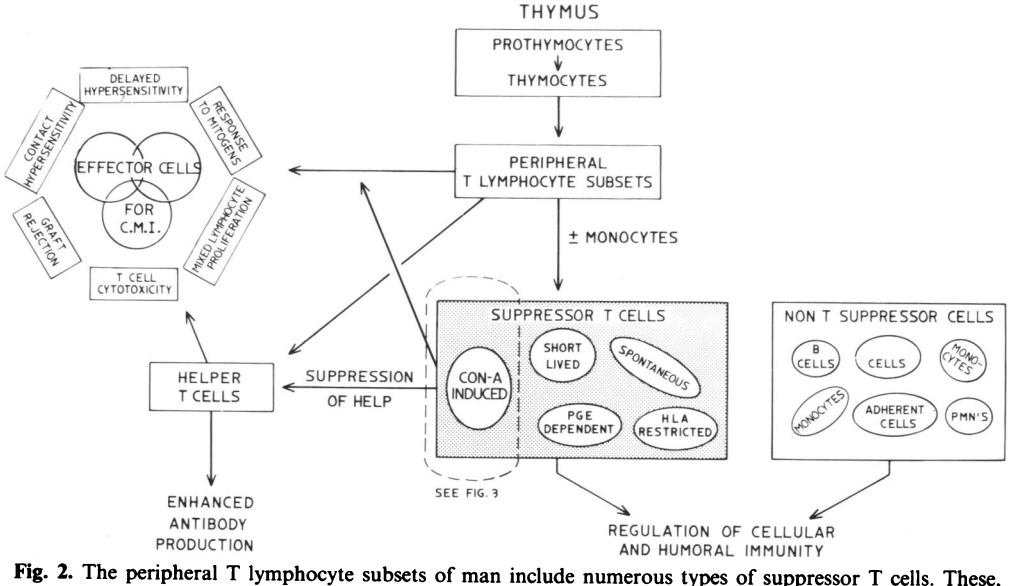

Fig. 2. The peripheral T lymphocyte subsets of man include numerous types of suppressor T cells. These, together with non-T cell suppressors, regulate both cellular and humoral immunity. One subpopulation of suppressor T cells is sensitive to concanavalin A.

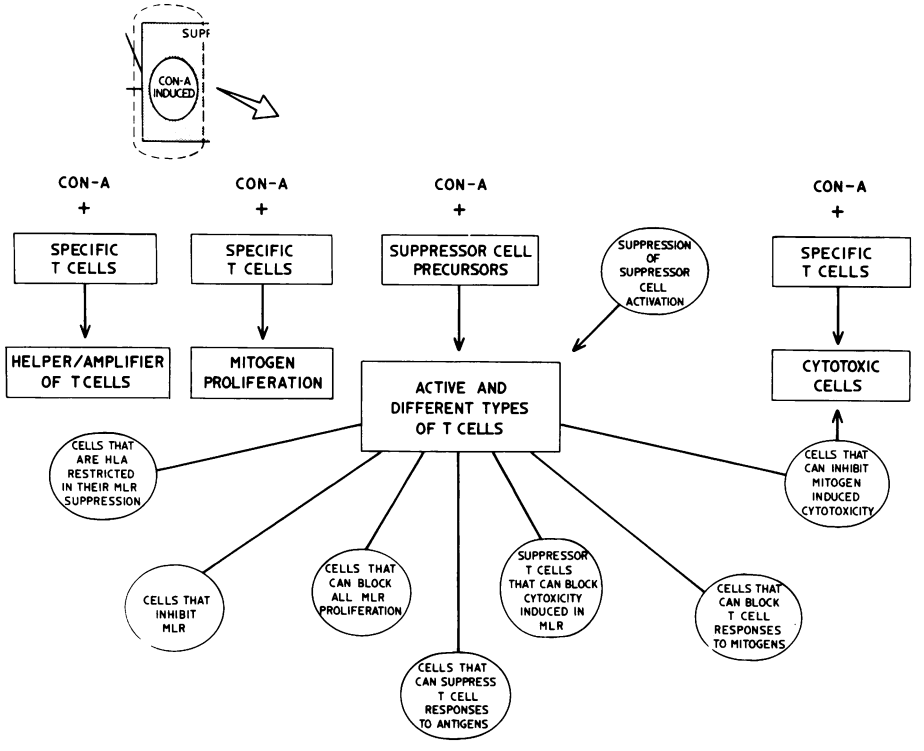

Fig. 3. Suppressor cell precursors that are activated by concanavalin A give rise to active and different types of suppressor cells that are distinguished in a functional sense. Such suppressor cells are able to exercise immunoregulatory activity over at least seven different T cell functions. While it is certain that there are different types of suppressor cells responding to concanavalin A, it is likely that there is a functional overlap on the part of a number of these cells. Note that apart from suppressor cell precursors giving rise to active suppressor cells, there is another order of immunoregulatory activity represented by the TH_2-negative cells that are capable of suppressing suppressor cell activation.

1977) include different inflammation-producing cells that can express the full range of cell-mediated immune responses, helper cells (Shore, Dosch & Gelfand, 1978) and suppressor cells.

Depending on the assay system used, apparently distinctive T suppressor cells have been identified in man although there is probably some overlap among cells identified in single-function studies. Spontaneously active (Bresnihan & Jasin, 1977), short-lived (Shou, Schwartz & Good, 1976; Dwyer, Johnson & Desaules, 1979), prostaglandin-producing (Pelus & Strausser, 1977; Webb & Nowowiejski, 1978) and suppressor cells whose function is HLA-restricted (Engleman & McDevitt, 1978; Engleman, 1978) co-exist with suppressor cells that can be activated by Con A. In addition, good evidence suggests that B cells (Neta & Salvin, 1976), null cells (Muchmore, Decker & Blaese, 1977) and adherent cells of both the polymorphonuclear (Hsu, Wu & Rivera-Arcilla, 1979) and more especially the monocyte/macrophage series can be immunosuppressive (Ptak & Gershon, 1975; Birnbaum & Swick, 1978).

As in the mouse, more than one subset of human peripheral blood lymphocytes is triggered by Con A (Fig. 3). So far, Con A-activated T cells have been associated with suppression of (1) the effectiveness of the lymphokine macrophage inhibition factor (Fox & Rajaraman, 1978); (2) the proliferation of responder cells in a mixed leucocyte culture (MLC) (Reinherz & Schlossman, 1979; Shou *et al.*, 1976); (3) the proliferation of HLA-D-specific responder cells in a mixed leucocyte culture (MLC) (Engleman & McDevitt, 1978); (4) T cell proliferative responses to soluble antigens (Shou *et al.*, 1976); (5) the cytotoxicity directed against allogeneic cells in the second phase of an MLC; (6) T cells proliferating in response to mitogens (e.g. PHA and Con A itself) (Hubert, Delespesse & Govaerts, 1976; Dwyer *et al.*, 1979); (7) mitogen-induced, T cell-mediated,

non-specific cytotoxicity (Hunninghake & Fauci, 1978); and (8) T cell proliferation in response to challenge with 'altered self' (e.g. trinitrophenylated autologous lymphocytes) (Mayumi *et al.*, 1979).

Most of the studies in man have used similar methods for inducing suppressor activity and measuring this in a responsive indicator system. Putative suppressor cells are stimulated with Con A, exposed to mitomycin C to prevent further DNA synthesis among these cells, washed and then added to autologous or allogeneic cells subsequently stimulated with mitogen or cell surface antigens. Control cultures feature identical cells not stimulated by Con A. Suppression is usually reported as the percentage reduction in the responsiveness of the indicator cells co-cultured with the Con A-stimulated cells when compared to that of indicator cells co-cultured with control cells. Many studies using this system or variations on this theme have revealed interesting aspects of human suppressor cell performance.

A wide range of concentrations of Con A obtained from a number of sources can activate suppressor activity. Various laboratories have used from 1 to 100 μg of Con A per 10^6 cells stimulated. While it is not clear if the same suppressor cell is activated in each case, it is obvious that doses used to produce a strong mitogenic response are not necessary for suppressor cell activation. Suppressor cells need only a brief exposure to Con A to be activated although for optimal suppressor cell activity a culture period of at least 12–24 hr may be necessary (de Gast *et al.*, 1977). It is of interest that induction of killer activity by Con A requires its continued presence (Asherson, Ferluga & Janossy, 1973). Con A appears to cross-link cell surface receptors in activating suppressor cells (Greaves & Janossy, 1972). It is possible, therefore, that Con A will be carried over into the indicator system. Most examinations of this possibility (Sakane & Green, 1977; Dwyer *et al.*, 1978), but not all (de Gast *et al.*, 1977), have concluded that in practice this does not occur to any significant extent. Mitomycin C treatment of activated suppressor cells does not affect their function indicating, as in the mouse, that DNA synthesis is not needed for active suppression (Shou *et al.*, 1976; de Gast *et al.*, 1977). Mitomycin C treatment of cells *before* their exposure to Con A blocks the generation of suppressor activity that can suppress B cell production of immunoglobulin and those suppressor cells specific for mitogen-induced cytotoxicity (Hunninghake & Fauci, 1978; Siegal & Siegal, 1977). These latter suppressor cells are not steroid-sensitive nor dependent on macrophages and do not die rapidly in culture (Hunninghake & Fauci, 1978). Con A-activated suppressor cells that modify responses to mitogens and allogeneic cells, on the other hand, can still be activated by Con A even after treatment with mitomycin C (Lobo & Spencer, 1979; Kurnick, Bell & Grey, 1976; Sakane & Green, 1977). Such observations strongly suggest that humans as well as mice have distinctive subpopulations of suppressor cells triggered by Con A.

Many studies have reported that cells able to respond to Con A *in vitro* and subsequently suppress mitogen and MLC reactivity, die rapidly in culture (Bresnihan & Jasin, 1977; Birnbaum & Swick, 1978; Feighery *et al.*, 1978). This conclusion is based on complementary evidence demonstrating that (a) successful generation of suppressor cells with normal cells precultured for 24 hr is difficult, while (b) proliferative responses to mitogens and antigens are increased by preculturing responder cells for 24 hr (Feighery *et al.*, 1978; Dwyer *et al.*,1979). After 24 hr in culture, however, the situation changes and suppressor cells are more readily activated. Even suppressor cells precultured for 8 days can be activated by Con A (Dwyer *et al.*, 1979). It seems likely that a considerable number of suppressor cells die the first 24 hr in culture, perhaps releasing transiently active suppressor substances into the culture medium that prevent activation by Con A of other suppressor cells. After this period the putative suppressor cells die slowly (Dwyer, unpublished observations). There is evidence to suggest that some suppressor cells go through cyclical periods of responsiveness and unresponsiveness to Con A (Dwyer *et al.*, 1979).

Low-density macrophages are probably necessary for optimal activation of suppressor cells by Con A (PHA-stimulated indicator system) (Raff, Cochrum & Stobo, 1978). Indeed, preferential activation of helper or suppressor cell mechanisms may depend to some extent on the way antigens are presented by macrophages (Shevach, 1976). Abnormalities of macrophage–T interactions may also serve as the basis of immunological hyporeactivity found in some clinical disorders such as Hodgkin's disease (Twomey *et al.*, 1975) and multiple myeloma (Broder *et al.*, 1975). However, suboptimal but definite suppressor cell induction follows Con A stimulation of highly purified T cells (Sakane & Green, 1977).

Con A-activated cells suppress T cell responses to mitogens equally well in a wholly autologous or allogeneic system (Shou *et al.*, 1976; Dwyer *et al.*, 1979). Con A-activated cells suppress the response of lymphocytes to soluble antigens and allogeneic cells equally as well as they suppress the response to mitogens (Shou *et al.*, 1976). Suppressor cells need not be present at the commencement of indicator cultures utilizing MLC or mitogen responses (de Gast *et al.*, 1977). Con A-activated suppressor cells added to an indicator culture 24 hr before its termination can provide significant suppression (Dwyer & Johnson, unpublished observations). Thus it seems likely that Con A-activated cells suppress DNA synthesis in susceptible cells rather than inhibiting the addition of more cells to the proliferative response. It is certain that suppression is not induced as a result of killing of responder cells (Shou *et al.*, 1976).

B cells are stimulated by Con A to undergo moderate proliferation and will, after treatment with Con A, stimulate an autologous mixed lymphocyte reaction. They do not, however, become suppressor cells (Sakane & Green, 1977). The suppressor cells that respond to Con A triggering are thymus-derived lymphocytes. Many of the characteristics of these cells have been discovered from density gradient studies and examination of the cell membrane receptors. Discontinuous bovine serum albumin (BSA) density gradient separation of T cells which have been activated by Con A (Sakane & Green, 1977) demonstrated that suppressor activity exists among populations that are both proliferating and not proliferating after stimulation with Con A. However, when density gradient separation of T cells was performed prior to incubation with Con A, cells which proliferated were found to have low density and exhibited no suppressor qualities while fractions containing high-density T cells produced marked suppression but incorporated very little thymidine (Sakane & Green, 1977; Glinski *et al.*, 1976). These studies confirmed previous data suggesting that proliferation was not a requisite for suppressor activity. It is of interest that density separation also suggested that T cells which suppress lymphocyte responses to mitogen may differ from those that suppress an MLC (Sakane & Green, 1977). Other reports show that mitogen-responsive suppressor T cells are found among the large cells recovered from the most rapid fractions of a velocity sedimentation apparatus (Winger, Nowell & Daniele, 1978).

T cells responding to Con A are undoubtedly heterogeneous. Information gained from the suppression of one indicator system may not apply to Con A-activated suppressor cells in general. Some human T cells carry a receptor for the Fc portion of IgG (T gamma cells) while others express a receptor for the Fc portion of IgM (T mu cells) (Moretta *et al.*, 1977). In assays measuring the release of antibodies by PWM-stimulated B cells, T gamma cells appear to be suppressor cells and T mu helper cells. The division is not unexpectedly relative rather than absolute (e.g. some cytotoxic T cells are found in the T gamma fraction (Grossi *et al.*, 1978).

The Con A-activated T cells which suppress antibody release are mainly T gamma cells (Moretta *et al.*, 1976, 1977). In man these cells and their precursors carry a receptor for theophylline (Limatibul *et al.*, 1978; Shore *et al.*, 1978). Neither characteristic has been adequately studied in Con A-activated systems which regulate T cells but cells that can suppress T cell responses to mitogen can be found in both T mu and T gamma fractions. A separation of lymphocytes on the basis of their affinity for histamine-coated beads revealed that the histamine-binding subpopulation of cells was enriched for Con A suppressor cells. Furthermore, it is known that histamine inhibits lymphocyte proliferation *in vitro* (Ballet & Merler, 1976; Rocklin, 1976). Treatment of human cells with anti-DR antisera and complement either prior to or after Con A activation does not interfere with the generation or expression of suppressor activity (Fineman, Mudawwar & Geha, 1979). Thus, DR-negative cells expressing a receptor for histamine may be similar to the I-J$^-$ Ly 2,3 suppressor cells in the mouse.

In man approximately 30% of peripheral T lymphocytes express an antigen—'TH$_2$' (Evans *et al.*, 1978). Most of the killer cell activity in cell-mediated lympholysis is mediated by the TH$_2^+$ subset. Such cells proliferate poorly when stimulated by allogeneic cells and not at all after co-culture with soluble antigens. T cells which after Con A activation can suppress an MLC are TH$_2^+$ (Reinherz & Schlossman, 1979). The TH$_2^-$ and TH$_2^+$ cells are programmed for their respective functions (help and suppression) independent of their ability to discriminate and react to non-specific polyclonal mitogens or antigens (Cantor, Shen & Boyse, 1976). The programming before cell activation of specific cell functions seems to be linked to the expression of a particular cell

surface phenotype. TH_2^- cells may well be regulators (or suppressors) of TH_2^+ suppressor cells. In purified preparations, TH_2^+ cells could be induced to suppress MLC reactions after 24 hr of co-culture with Con A. The unseparated T cell population required 48 hr of co-culture with Con A to be similarly active (Reinherz & Schlossman, 1979). The TH_2^- subset was shown to regulate the activity of TH_2^+ suppressor cells. The failure of Con A to activate suppressor T cells in disease states will require examination of both the TH_2^- and TH_2^+ cells as excessive TH_2^- suppression of suppressor cells could result in disease. In a similar indicator system, Rice, Laughter & Twomey (1979) found the Con A-responsive T cell to be non-adherent, short-lived in culture and slightly sensitive to irradiation. Autologous lymphocytes responding to altered self (TNP-autologous lymphocytes) can be suppressed by Con A-activated T cells that are resistant to 2,000 rad after activation (Mayumi *et al.*, 1979). Once activated by Con A, suppressor T cells that suppress T effector cells in contradistinction to those that suppress B cells are resistant to steroids (Fauci, Pratt & Whalen, 1977; Dwyer *et al.*, 1979). Preincubation of lymphocytes with dexamethasone or hydrocortisone before activation by Con A can significantly reduce the triggering by this mitogen of suppressor cells that block mitogen responses (Dwyer *et al.*, 1979; Knapp & Posch, 1980). How Con A-activated T cells suppress T cell responses is unknown. Soluble factors from T cells which suppress B cell responses can be readily demonstrated in supernatants but such factors have not significantly suppressed T cell responses. PHA responses were suppressed in one study by Con A-activated cells separated from responder lymphocytes by millipore filter. No suppression occurred, however, when the cells were separated by a dialysis membrane (10,000 mol. wt limit) (Winter *et al.*, 1978; Hirano & Nordin, 1976).

Lymphocytes from 12–15% of apparently normal healthy humans will not suppress mitogen or MLC responses after co-culture with Con A (Dwyer *et al.*, 1979; Shou *et al.*, 1976; Birnbaum & Swick, 1978). This phenomenon is of considerable importance if the assay is to be applied to clinical studies where failure to respond to Con A could be erroneously attributed to disease. The well-characterized high-responder or low-responder status found in inbred strains of mice and guinea-pigs (Benacerraf & Katz, 1975) is more difficult to detect in outbred humans but has been adequately documented in both humoral and cell-mediated immune systems (Haynes & Fauci, 1978). As in animals, low-responder status among humans appears to be actively maintained by suppressor T cells. Humans who develop few plaque-forming cells (PFC) after challenge with SRBC have suppressor cells rather than effector cells that differ from those who develop many more PFC (Haynes & Fauci, 1979). The development of cytotoxic T cells among lymphocytes stimulated by Con A segregate consistently into high and low responders (Hunninghake & Fauci, 1977, 1978). Convincing evidence suggests that the ease of induction of cytotoxicity is related to variations in suppressor cell control of the reaction. Responses are not HLA-related and the high- and low-responder status has not as yet been demonstrated in studies of lymphocyte proliferation. It is possible, however, that failure of certain humans to develop suppressor cell activity after Con A treatment could be related to high- and low-responder status of the TH_2^- type cells that influence suppressor cells. These influences would presumably be consistent in one individual. In examining this question (Dwyer *et al.*, 1979) did not find any normal individuals who consistently failed to develop suppressor cell activity after stimulation with Con A. In fact, it seems more likely that variation was due to cyclical changes in the responsiveness of cells exposed to Con A. In assays simultaneously comparing suppressor cell activity generated by Con A in both 3 and 4 days of culture, no normal subjects were found who did not express good suppressor cell activity on one of the 2 days (Dwyer *et al.*, 1979). It thus seems unlikely that failure to respond at one time point will have prognostic significance with regard to the future development of the immunopathology related to defects in immunoregulation (Shou *et al.*, 1976). Another cause for an apparent lack of suppressor cell activity in the Con A assay is significant spontaneous (*in vivo*) induced suppressor cell activity. This results in suppression of the control cultures that can be little enhanced by the Con A-activated cells (Dwyer & Johnson, unpublished observations).

There is a need for standardization of procedures that will allow comparison of the results obtained in different laboratories. Without this there is no way to be confident that the same suppressor cells performing the same function are being examined. Obviously, the fact that suppressor cells can be activated by Con A does little to reduce the variables being studied.

Table 1. Summary of assay systems and clinical studies relating to the immunoregulation of T cells

Disease or state	Generation of suppressor cells	Indicator system	Suppressor cell activity	Comments	Reference
Hodgkin's disease	Spontaneous	MLC	Increased	Monocyte and T cell suppressors	Hillinger & Herzig (1978)
	Spontaneous	MLC	Increased	Reversed in remission	Twomey *et al.* (1975)
	Spontaneous	PHA	Increased	Glass wool-adherent	Sibbitt *et al.* (1978)
	Spontaneous	PHA	Increased	PGE_2 producing suppressor cells	Goodwin *et al.* (1977)
Chronic lymphocytic leukaemia	Spontaneous	PHA	Increased	T helper cells normal	Faguet (1979)
Smoking and pregnancy	Spontaneous	PHA	Decreased	Not a serum factor	Paganelli *et al.* (1979)
Cord blood	Spontaneous	MLC	Increased	Not cytotoxic	Olding & Oldstone (1974)
Leprosy	Lepromin-induced	Con A	Increased	Lepromatous leprosy	Mehra *et al.* (1979)
Tuberculosis	PPD-induced	PPD-induced	Increased	Subset of patients anergic	Ellner (1978)
Chronic graft-*vs*-host disease	Spontaneous	MLC	Increased	After marrow grafting	Tsoi *et al.* (1979)
Schistosomiasis	Schistosomal antigen induced	PHA	Increased	Con A-activated suppressor cells normal	Colley *et al.* (1978)
Filariasis	Filarial antigen	Proliferation to filarial antigen	Increased	Factors abnormal	Piessens *et al.* (1980)

Burn patients	Spontaneous	MLC	Increased	Suppressor cell active in patients, not the PHA responsiveness	Miller & Baker (1979)
Multiple sclerosis	Con A	Con A	Decreased	Just before exacerbation	Arnason & Antel (1978)
Graves' disease	Con A	Con A	Decreased	Related to B8	Balazs *et al.* (1979)
Inflammatory bowel disease	Con A	Con A	Decreased	Severe and active disease	Hodgson *et al.* (1978)
Myasthenia gravis	Con A	Con A	Decreased	B8, DR3-related	Zilko *et al.* (1979)
SLE	Con A	PHA	Decreased	T gamma cells low	Newman *et al.* (1979)
	Con A	MLC	Decreased	70% of patients	Kaufman & Bostwick (1979)
	Con A	Delayed Con A	Decreased	Reversal by steroids	Bresnihan & Jasin (1977)
Candidiasis endocrinopathy syndrome	Con A	PHA	Decreased	Family study	Arulanantham *et al.* (1979)
Idiopathic thrombocytopenic purpura	Con A	PHA	Decreased	Active disease in children	McIntosh *et al.* (personal communication)
Old age	Con A	PHA	Decreased	Responder cells more susceptible to suppressor activity	Antel & Arnason (1979)

Nevertheless, the assay systems described above have been applied in many clinical studies and some of the more important are tabulated and referenced in Table 1. In addition, some of the other clinical studies relating to the immunoregulation of T cells are included. In a number of clinical studies, assays of suppressor cell performance have been consistently abnormal and related well to immunological phenomena observed *in vivo*. Assays using Con A-stimulated suppressor T cells are not suitable for detecting enhanced suppressor cell activity. However, an expansion of the work on spontaneous or non-Con A-activated suppressor cell activity may soon overcome this problem. There seems no reason to doubt that more information about human immunoregulatory cells will be supplied by the Con A stimulation assay. Such studies combined with advances in our ability to examine antigen-specific suppression will predictably lead to enhanced understanding of clinical situations and ways of deliberately manipulating suppressor cells, the current 'anti-heroes' of immunopathogenesis.

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